Polar Biol (2015) 38:2047–2058
DOI 10.1007/s00300-015-1765-1

ORIGINAL PAPER

Widespread intra-specific genetic homogeneity of coastal Antarctic benthic foraminifera

Wojciech Majewski[1] · Samuel S. Bowser[2] · Jan Pawlowski[3]

Received: 20 November 2014 / Revised: 1 June 2015 / Accepted: 23 July 2015 / Published online: 18 August 2015

Abstract Benthic foraminifera are a major component of the Antarctic biota. Coastal foraminiferal morphospecies are widely distributed in Antarctic waters. The question is whether these morphotypes are genetically identical or, rather, they represent a cohort of cryptic species. Here, we compared genetically nine benthic foraminiferal morphospecies from Admiralty Bay (South Shetlands) and the western Ross Sea (McMurdo Sound, Terra Nova Bay), separated by a distance of ~4500 km. Additionally, for three of these morphospecies, we included specimens from Rothera (Marguerite Bay), which is located between the two main areas of interest. Our study, based on SSU and ITS rDNA sequence data, shows that all examined morphospecies share the same genotypes despite the presence of considerable intra-individual genetic variability.

Keywords Foraminifera · Biogeography · SSU rDNA · Molecular diversity · Protists

Electronic supplementary material The online version of this article (doi:10.1007/s00300-015-1765-1) contains supplementary material, which is available to authorized users.

✉ Wojciech Majewski
wmaj@twarda.pan.pl

[1] Institute of Paleobiology PAS, Twarda 51/55, 00-818 Warsaw, Poland

[2] New York State Department of Health, Wadsworth Center, Albany, NY, USA

[3] Department of Genetics and Evolution, University of Geneva, Geneva, Switzerland

Introduction

Benthic foraminifera from Antarctic seas have been studied using morphological approach for more than a century. Chapman and Parr (1937), Heron-Allen and Earland (1922, 1932), Earland (1934, 1936), Parr (1950), and Wiesner (1931) are among the most substantial of early contributions; for a detailed review see Gooday et al. (2014). As a result of these efforts, sufficient data exist for analyzing the biogeography of the most typical morphospecies. A broad distribution of some Antarctic morphospecies was suggested by some early foraminiferal workers (e.g., Earland 1934) and more recently confirmed by Mikhalevich (2004), who concluded that the majority of foraminiferal morphospecies on the Antarctic shelf have circumpolar distributions. The idea is based, however, exclusively on morphological studies, which have been shown to underestimate the true foraminiferal diversity revealed in many cases by genetic data (e.g., de Vargas et al. 1999; Holzmann 2000; Pawlowski et al. 2002b, 2008; Habura et al. 2004).

Over the last two decades, molecular approach was used to test hypotheses regarding the circum-continental distribution of various Antarctic organisms. In many cases, cryptic species were discovered, e.g., among amphipods (Held 2003; Held and Wagele 2005; Lörz et al. 2009; Baird et al. 2011, 2012), isopods (Raupach and Wägele 2006), ostracods (Brandão et al. 2010), sea spiders (Mahon et al. 2008), bivalves (Guidetti et al. 2006; Linse et al. 2007), sea stars (Janosik and Halanych 2010), crinoids (Wilson et al. 2007; Hemery et al. 2012), and benthic octopus (Allcock et al. 1997; Strugnell et al. 2009). By contrast, other organisms were confirmed to exhibit a circumpolar distribution, e.g., two species of shrimp (*Chorismus anatrcticus* and *Nematocarcinus lanceopes*) that recolonized Antarctic

coastal waters following late Pleistocene climatic oscillations (Raupach et al. 2010).

Until now, genetic studies of Antarctic foraminifera were conducted on material from shallow-water habitats of the western Ross Sea in McMurdo Sound (Bowser et al. 2002; Pawlowski et al. 2002a), Terra Nova Bay (Sabbatini et al. 2004), King George Island's Admiralty Bay (Sinniger et al. 2008; Majewski and Pawlowski 2010; Pawlowski and Majewski 2011), as well as from deep-sea settings of the Weddell Sea (Gooday et al. 2004; Gooday and Pawlowski 2004; Cedhagen et al. 2009). A great majority of these investigations explored single-chambered (monothalamous) foraminifera. Rotaliid sequences from the same areas were also utilized in some general phylogeographic and taxonomic studies (Lecroq et al. 2009; Schweizer et al. 2005, 2009, 2012).

Sequencing of individual foraminifera allowed broader biogeographic investigations. Molecular studies of four morphotypes of shallow-water allogromiid foraminifera showed the presence of separate genotypes or cryptic species in the Arctic and Antarctic, questioning the presence of true bipolarity among these organisms (Pawlowski et al. 2008). These results contrasted sharply with observations from the deep sea, where gene flow between Antarctica and the Arctic was documented in three common abyssal polythalamous calcareous species (Pawlowski et al. 2007b). A genetic comparison of individual specimens of the calcareous species *Epistominella* sp. collected from shallow-water (15–30 m) sites in McMurdo Sound in the Ross Sea with those from deep (>1000 m) settings in the Weddell Sea turned out to be especially noteworthy. Their strong genetic similarity (Pawlowski et al. 2007a) provided the first genetically documented example of a eurybathyal foraminiferal species to inhabit very distant areas of Antarctica, suggesting that Antarctic foraminiferal genotypes may be much more widely distributed than currently believed.

In the present study, we tested the genetic similarity of nine common benthic foraminifera (Figs. 1, 2) from very distant coastal areas of West Antarctica, i.e., Admiralty Bay on King George Island (South Shetlands) and the Ross Sea, mostly from McMurdo Sound at its SW limits (Fig. 3). This comparison is supplemented by molecular data from Marguerite Bay near Rothera station. In all cases, we found evidence for genetic similarity of these species. Because of the wide geographical distances and environmental differences between our major sampling areas, our findings may extrapolate to most coastal areas of West Antarctic, if not around the entire Antarctic continent.

Methods

Sampling

Samples from McMurdo Sound and Terra Nova Bay (Ross Sea) were collected during several field seasons between 1998 and 2011. Individual specimens were extracted from sediment surface samples obtained either by an airlift sampler (Pollock and Bowser 1995) or from cores collected

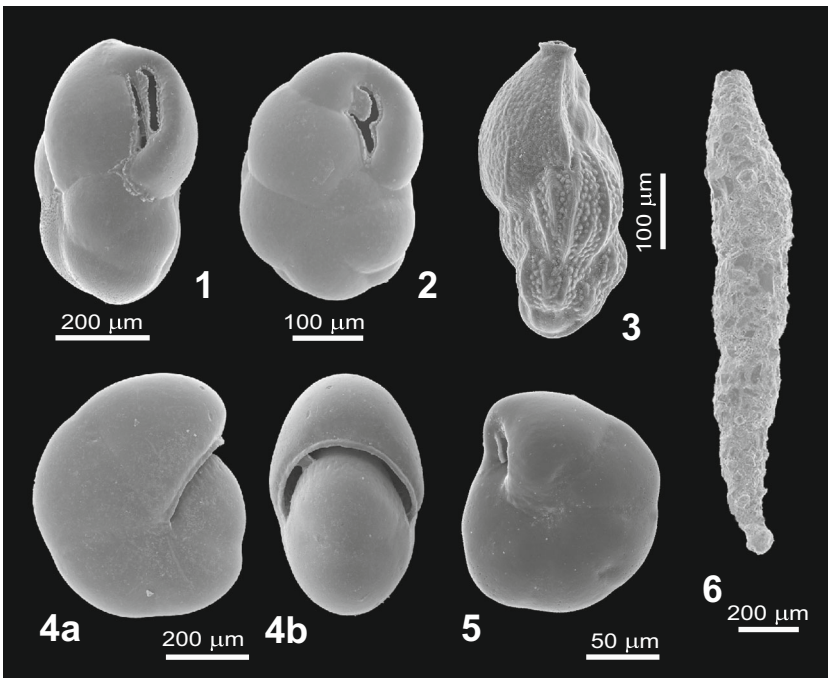

Fig. 1 Multi-chamber benthic foraminifera investigated in this study: *1–2 Globocassidulina biora*; *3 Trifarina earlandi*; *4 Pullenia subcarinata*; *5 Epistominella* sp.; *6 Reophax* sp. All specimens are from Admiralty Bay (South Shetlands). For more information see Majewski (2005, 2010)

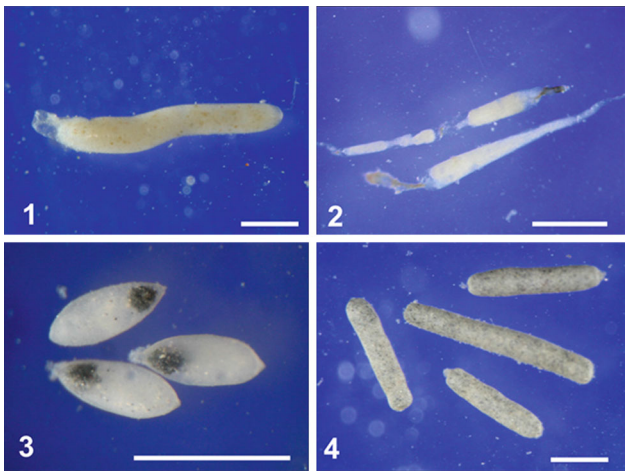

Fig. 2 Monothalamous foraminifera investigated in this study: *1 Bowseria arctowskii*; *2 Micrometula* sp.; *3 Psammophaga magnetica*; *4 Hippocrepinella hirudinea*. *Scale bars* = 0.5 mm. All specimens are from Admiralty Bay (South Shetlands). For more information see Majewski et al. (2007) and Sinniger et al. (2008)

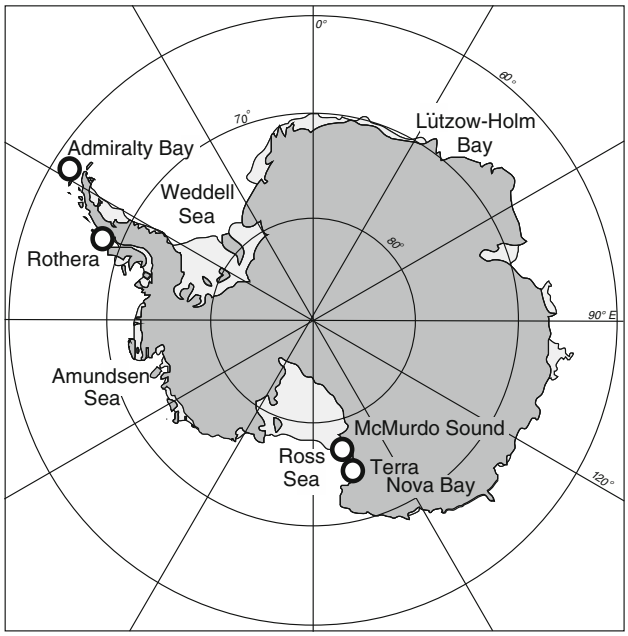

Fig. 3 Map showing sampling localities

by divers. Samples from Admiralty Bay were collected in early 2007 using a shipboard-operated Van Veen sampler and a core sampler, as well as handheld cores taken by divers. Individual specimens were isolated from the upper 2–3 cm of surface sediments. Samples from the Ross Sea and Admiralty Bay were processed immediately after recovery. They were gently washed over a set of sieves with cold seawater and stored for a few days at ~2 °C. Samples from Rothera were collected in 2013 by divers or with a grab. They were transported frozen and stored at −80 °C in the Department of Genetics and Evolution, University of Geneva. Before searching for individual specimens, parts of samples were unfrozen and washed gently over a 125-µm sieve. The >125-µm residues were scanned for allogromiid and hard-shelled foraminifera with visible cytoplasm. The specimens were transferred individually into guanidine or AP1 (DNAEasy, Qiagen) extraction buffer. Details on sampling sites for all 133 analyzed foraminiferal specimens are provided in Online Resource 1.

DNA extraction, PCR, cloning, and sequencing

DNA was extracted in guanidine lysis buffer (Pawlowski 2000). Most extractions were performed with a single specimen (Online Resource 1). PCR amplifications of a fragment of the SSU rDNA were performed using the primer pair s14F3 (5′ACG CA(AC) GTG TGA AAC TTG) and newB (5′ TGC CTT GTT CGA CTT CTC). PCR products were reamplified using the nested primer s14F1 (5′AAG GGC ACC ACA AGA ACG C) and primer newB. The complete ITS rDNA region was amplified using universal eukaryotic primer s20 (50-TTG TAC ACA CCG CCC GTC) and reamplified with primer sBr (5′-GTA GGT GAA CCT GCA GAA GG) situated at the 3′ end of the SSU rDNA and the foraminiferal-specific primer 2TAIC (5′-CTC ACT CGA GCT GAT GTG) situated at the 5′ end of the LSU rDNA (according to Pawlowski et al. 2007a). The amplified PCR products were purified using a High Pure PCR Purification Kit (Roche Diagnostics). After purification, positive PCR products were ligated in the Topo TA Cloning vector (InvitroGen, Basel, Switzerland) or in the pGEM-T vector system (Promega, Duebendorf, Switzerland), and cloned using One Shot TOPO10 chemically competent cells (InvitroGen). Sequencing was done with an ABI PRISM BigDye Terminator Cycle Sequencing Kit using an ABI 3130XL DNA sequencer (Applied Biosystem, Rotkreuz, Switzerland), according to the manufacturer's instructions. New sequences reported in this paper were deposited in the EMBL/GenBank database.

Molecular data analysis

In total, 118 isolates were analyzed for SSU, providing 256 sequences of nine morphospecies (Table 1). Nineteen of these isolates representing three morphospecies were also analyzed for ITS, yielding 69 additional sequences (additional morphospecies were not analyzed for ITS due to amplification problems). This dataset was supplemented by two sequences of *Epistominella* sp. from the Weddell Sea (Pawlowski et al. 2007a). The sequences were aligned using Seaview 4.4 (Gouy et al. 2010). Sequence divergence

Table 1 SSU and ITS rDNA from common foraminiferal morphospecies from the Ross Sea (RS), Admiralty Bay (ADM), and Rothera (ROT)

Species	Gene	RS	ADM	ROT
Globocassidulina biora	SSU	6/18	8/11	3/9
Trifarina earlandi	SSU	6/26	6/25	
	ITS	3/8	3/7	
Pullenia subcarinata	SSU	5/10	6/14	
	ITS	4/9	4/10	
Epistominella sp.	SSU	7/9	5/11	
Bowseria arctowskii	SSU	2/7	7/7	
Reophax sp.	SSU	5/12	4/11	
	ITS	2/13	3/22	
Micrometula sp.	SSU	9/9	4/7	88/24
Psammophaga magnetica	SSU	6/9	9/10	
Hippocrepinella hirudinea	SSU	5/12	4/7	3/8

Number of isolates (1st number) and clones (2nd number) are indicated

was calculated with BioEdit 7.2.5 (Hall 1999) as the ratios of differences to the length of the longer of the two sequences. Relationships within the morphospecies were calculated with haplotype networks using the Median-Joining algorithm in Network 4.612 (Röhl 2002). This method allows simple reconstruction of phylogenies based on intra-specific genetic data (e.g., González-Wevar et al. 2012). The haplotype matrices were prepared manually with all indels, regardless of their lengths, considered as single events.

Morphological analysis

Comparison of morphotypes/morphospecies of benthic foraminifera found in McMurdo Sound and Admiralty Bay was based on the authors' collections and the literature. The analysis was limited to the two primary study areas, as they are roughly comparable in size and habitat diversity and were both thoroughly investigated for benthic foraminifera. Only publications with detailed photographic documentation were used for the analysis, i.e., Gooday et al. (1996) and Ward (1984) for McMurdo Sound, and Majewski (2005, 2010) and Majewski et al. (2007) for Admiralty Bay. They were supported in some respects by the work of Bernhard (1987), Bowser et al. (2002), DeLaca et al. (2002), Majewski and Pawlowski (2010), Pawlowski and Majewski (2011), Schweizer et al. (2012), and Sinniger et al. (2008). Percentages of morphospecies shared between the two areas were calculated in relation to the total number of species found at both locations.

Results

Morphospecies comparison between McMurdo Sound and Admiralty Bay

According to our comparison of benthic foraminiferal taxa reported from McMurdo Sound and Admiralty Bay (Online Resource 2), 29.6 % of allogromiid morphospecies are common between the two areas (Table 2). The percentage of shared morphotypes is similar among lagenids (34.8 %) and miliolids (38.1 %). The percentage of common species is markedly higher among multichambered taxa (textulariids and rotaliids), numbering 57.6 and 66.7 %, respectively. In total, 41.6 % of benthic foraminiferal morphospecies are shared between McMurdo Sound and Admiralty Bay.

Molecular data

The SSU sequence divergence within and between the three sampling areas of our study are quite different for various morphospecies (Table 3). In five out of nine foraminifera analyzed, i.e., *Psammophaga magnetica*, *Bowseria arctowskii*, *Reophax* sp., *Pullenia subcarinata*, and *Epistominella* sp., SSU dissimilarities within and between different areas are of similar, very low values up to ~1 %. In three species, ITS shows similar patterns but

Table 2 Numerical comparison of benthic foraminiferal species/morphospecies in McMurdo Sound (the Ross Sea) and Admiralty Bay (South Shetlands)

	McMurdo Sound	Admiralty Bay	Common species
Allogromiid and monothalamous agglutinated	41	51	21 (29.6 %)
Textulariids	25	27	19 (57.6 %)
Spirillinids	3	1	1 (33.3 %)
Miliolids	12	17	8 (38.1 %)
Lagenids	42	20	16 (34.8 %)
Robertinids	1	1	1 (100 %)
Rotaliids	25	20	18 (66.7 %)
Total	149	137	84 (41.6 %)

Percentages of common species are calculated in relation to total number of species found at both locations

Table 3 Genetic dissimilarities in percentages within populations from Ross Sea (RS), Admiralty Bay (ADM), Rothera (ROT), and between the areas

Species	Gene	RS	ADM	ROT	RS/ROT/ADM
Globocassidulina biora	SSU	0.0–1.7	0.0–1.1	0–1.1	0.0–1.8
Trifarina earlandi	SSU	0.0–1.9	0.0–1.8		0.0–1.9
	ITS	0.8–4.8	0.2–2.4		1.2–4.8
Pullenia subcarinata	SSU	0.2–1.0	0.0–0.9		0.0–1.1
	ITS	0.7–2.2	0.0–2.1		0.2–2.6
Epistominella sp.	SSU	0.0–0.8	0.0–0.6		0.1–0.7
Bowseria arctowskii	SSU	0.2–0.5	0.0–0.2		0.0–0.4
Reophax sp.	SSU	0.0–0.3	0.0–0.7		0.0–0.5
	ITS	0.0–2.9	0.0–3.6		0.0–3.6
Micrometula sp.	SSU	0.0–0.4	0.0–0.7	0.0–11.9	0.0–11.8*
Micrometula sp. type I	SSU	0.0–0.4	0.0–0.7	0.5–1.4	0.0–1.2
Psammophaga magnetica	SSU	0.0–1.1	0.0–0.6		0.0–1.0
Hippocrepinella hirudinea	SSU	0.0–5.9	0.0–0.8	0.0–4.3	0.0–6.0*
H. hirudinea type I	SSU	0.0–0.8	0.0–0.8	0.0	0.0–1.1

* Taxa with multiple species

with divergences being at up to ~5 %. In two other cases, i.e., *Globocassidulina biora* and *Trifarina earlandi*, the SSU interregional dissimilarities approach 2 %, but they are not significantly larger than within a single sampling area. Finally, in the remaining two cases, i.e., *Hippocrepinella hirudinea* and *Micrometula* sp., the interregional divergences are significantly greater than 2 % and roughly an order of magnitude greater than within local populations. The patterns briefly described above are confirmed by haplotype networking (Figs. 4, 5). These networks show variable patterns within different morphotypes ranging from single clusters to multiple clusters separated by different genetic distances.

Discussion

Genetic diversity of Antarctic benthic foraminifera

Numerous genetic studies revealed cryptic species within planktonic (e.g., de Vargas et al. 1999; Darling and Wade 2008), rotaliid (e.g., Holzmann 2000), and monothalamous foraminifera (e.g., Habura et al. 2004; Pawlowski et al. 2008). Similarly, high hidden diversity within Antarctic planktonic *Neogloboquadrina pachyderma* sinistral (Darling et al. 2004) as well as among monothalamids was revealed using a single-cell sequencing approach (Pawlowski et al. 2002b) and by probing environmental DNA (eDNA) extracted from coastal or deep Southern Ocean sediments (Habura et al. 2004; Pawlowski et al. 2011; Lejzerowicz et al. 2014). In this study, we tested genetic similarity of common benthic foraminifera from very distant coastal areas of West Antarctica.

In our dataset, the SSU sequence divergence within and between the three sampling areas varies for different morphospecies (Table 3). In five cases, i.e., *P. magnetica*, *B. arctowskii*, *Reophax* sp., *P. subcarinata*, and *Epistominella* sp., the divergence values were very low (0.0–1.2 %). All these species show very similar SSU sequences for their Ross Sea, Admiralty Bay, and Rothera populations as well as in the interregional comparisons (Table 3), suggesting the presence of uniform species of broad geographic range and no significant restriction to gene flow.

In *G.biora* and *T. earlandi*, SSU sequences within and between different areas of our study were still similar, but their divergence was higher reaching up to 1.8 and 1.9 %, respectively (Table 3). A degree of genetic structure may be noted in *G. biora*, which shows considerable clustering (Fig. 4g). This point is clearer in *T. earlandi*, where SSU sequences cluster in two sister clades (Fig. 4h). The genetic variability within the later species clearly reflects intraspecific polymorphism. Four sequences of cloned amplicons obtained from a single isolate 7991 from Admiralty Bay (Online Resource 1) fall into both sister clades and show SSU sequence divergence up to 1.2 %, reflecting the largest intra-individual genetic variability within our dataset.

More genetic diversity was shown within the last two foraminiferal morphospecies examined, i.e., *H. hirudinea* and *Micrometula* sp. This variability is exhibited by SSU sequence divergence values up to 6.0 % in the first and up to as much as 11.8 % in the second morphospecies (Table 3). Multiple clades are also shown by haplotype networking (Figs. 5a), pointing to the presence of hidden diversity within these two morphospecies. In both cases, one large clade (type I on Fig. 5a, d) comprises the sequences from all three regions, while the other clades group haplotypes restricted to a single sampling area.

These observations are supported by analysis of ITS rDNA sequences that are more variable and can be used for

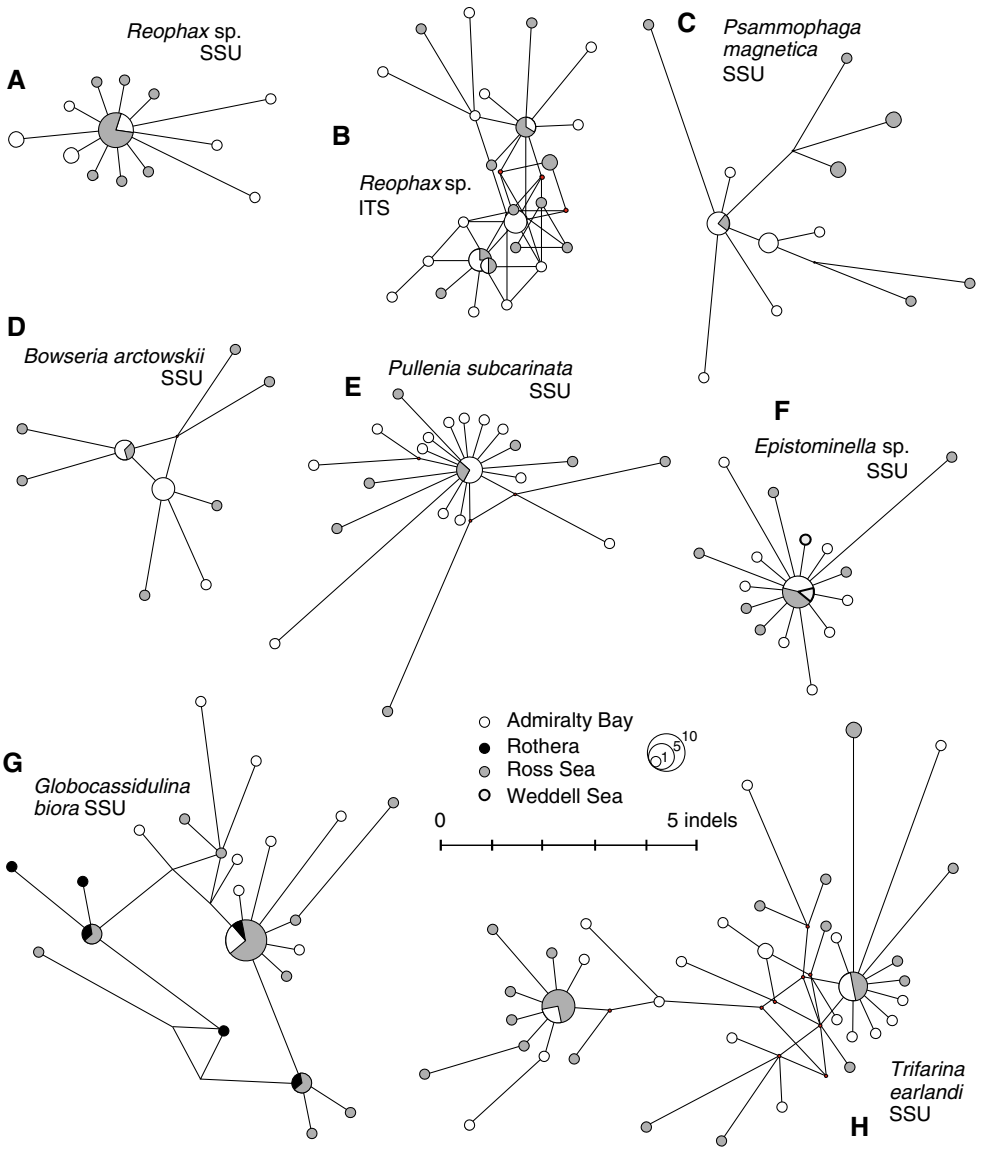

Fig. 4 Haplotype networks constructed using SSU and ITS sequences from Antarctic benthic foraminifera with little interregional genetic divergence. The area of *circle* is proportional to haplotype frequency. *Shades* of *gray* represent different locations

detection of cryptic species (e.g., Pawlowski et al. 2007a). In our study, *T. earlandi*, *Reophax* sp., and *P. subcarinata*, show analogous clustering patterns in ITS (Fig. 5b, c) as well as in SSU with markedly higher divergence values but not surpassing 5 % (Table 3).

Are shallow-water benthic foraminifera circum-Antarctic in distribution?

Until now, the hypothesis regarding the circum-continental distribution of typical Antarctic foraminifera was based exclusively on morphological data (e.g., Mikhalevich 2004; Gooday et al. 2014). For example, among all hard-shelled benthic foraminiferal morphospecies identified in Pine Island Bay in the central coast of West Antarctica, more than a half were also reported from the South Shetlands, the Ross Sea, and Lützow–Holm Bay in East Antarctica (Majewski 2013). Our comparison of foraminiferal morphospecies reported from McMurdo Sound and Admiralty Bay, showing that 41.6 % of all reported morphospecies are shared between the two distant areas, is consistent with these reports. The proportion of common species ranges between one-third and two-thirds, depending on which higher-level taxonomic groups are considered (Table 2), confirming strong morphological similarities of foraminiferal fauna around coastal Antarctica.

The most striking of our molecular results is the broad geographic distribution among benthic foraminiferal genotypes inhabiting shallow-water settings of West Antarctica. Among 12 of the genotypes recognized within nine foraminiferal morphospecies, nine are shared between the South Shetlands and the Ross Sea. The two areas are

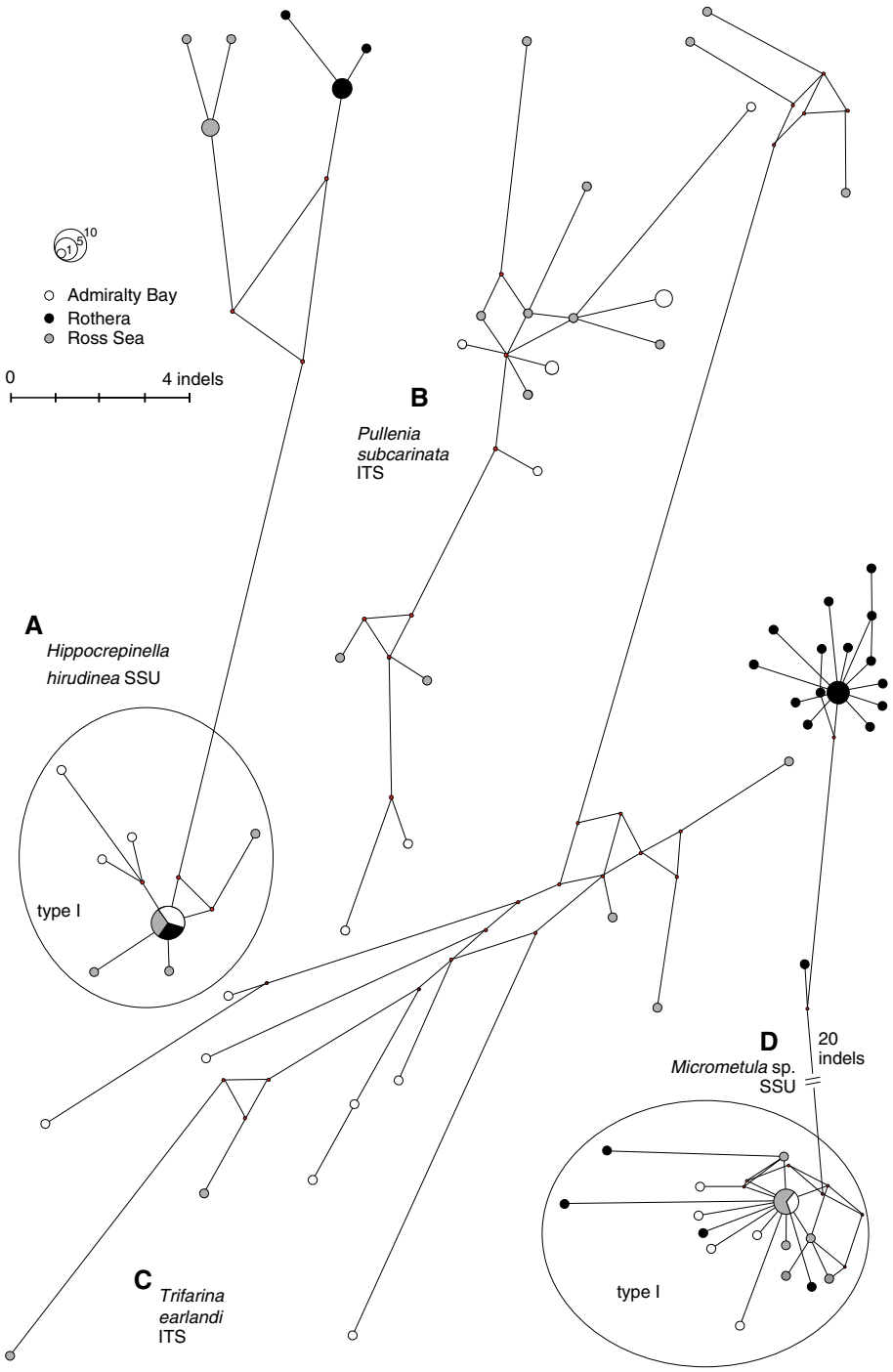

Fig. 5 Haplotype networks constructed using ITS and SSU sequences from Antarctic benthic foraminifera with considerable interregional genetic divergence. The area of *circle* is proportional to haplotype frequency. *Shades* of *gray* represent different locations. The ovals on graphs A and D mark widely distributed clades within *H. hirudinea* and *Micrometula* sp. referred to as type I

not only ~4500 km apart but are also of significantly different environments. Although both major areas of our study are located south of the Antarctic Convergence, the South Shetlands are considered to be the warmest region in Antarctica (King et al. 2003), while the waters of McMurdo Sound are among the coldest because of the extremely high latitude (78°S) and close proximity to the Ross Ice Shelf (Jacobs et al. 1979).

Although in McMurdo Sound, during the brief summer, the seawater temperature occasionally increases to −0.5 °C (Hunt et al. 2003; Cziko et al. 2014), seawater is believed to be perennially at or near its freezing point (~−2 °C). East McMurdo is characterized by an annual breakout of sea ice, and it is influenced by currents trending from the Ross Sea that during the summer are loaded with phytoplankton (Gooday et al. 1996 and references therein). On

the other hand, Explorers Cove in West McMurdo is bathed by cold currents from under the Ross Ice Shelf and shows multi-annual ice cover. Atmospheric precipitation, glacial melt, and runoff are very limited.

In contrast, the South Shetlands are significantly milder. During summer, in the bays of King George Island, a well-defined freshened uppermost layer of the water column may develop with temperatures up to 2 °C (Sarukhanyan and Tokarczyk 1988). It can also be loaded with suspended terrigenous material derived from glacial meltwater and runoff (Griffith and Anderson 1989). During the summer, deeper parts of the bays of King George Island are influenced by deep water with positive temperatures (Schloss et al. 2012), whereas these waters in winter are isothermal at ~−1.5 °C (Khim et al. 2007). Admiralty Bay experiences a relatively warm and humid climate regime with moderately high snow and glacial melting and runoff (Reynolds 1981). Due to air warming, strong winds, and intrusions of warm water masses from Bransfield Strait, the winter freezing of the bay is very variable and quite often it remains ice-free throughout the year (Kruszewski 2002).

Taking into account environmental differences between the Ross Sea and South Shetlands, it seems surprising that the shallow-water benthic foraminiferal species show so many similarities, not only at the morphological level (Table 2) but also genetically (Table 3). Because the two major areas of our study are as different ecologically as they could be around Antarctica and ~4500 km distant to each other, it seems likely that the nine morphospecies analyzed genetically are of circum-Antarctic distribution, i.e., are potentially spread throughout shallow-water coastal Antarctic habitats.

The possible influence of bathymetry in dispersal patterns

Our molecular results from Rothera add additional complexity to the distribution patterns of benthic foraminifera along the West Antarctic shelf. Marguerite Bay, situated between the South Shetlands and the Ross Sea, is approximately intermediate in terms of its environmental conditions. Our study sites near Rothera also differ in their deeper bathymetries and sediment characteristics, as well as in methods of sample processing. As a consequence, we collected there genetic material for only three out of nine morphospecies that are common between Admiralty Bay and the Ross Sea (i.e., *G. biora*, *H. hirudinea*, and *Micrometula* sp.).

Globocassidulina biora, collected from water depths of 8 and 515 m in Rothera, is genetically consistent with other areas (Fig. 4g). By contrast, among the two distinct genotypes of *H. hirudinea* collected in Rothera, one is identical to the Ross Sea and Admiralty Bay samples, but the other is significantly different (Fig. 5a). Similarly, we found that *Micrometula* sp. collected from 30 to 150 m at Rothera is genetically comparable to Ross Sea and Admiralty Bay specimens, but another genotype recovered from slightly deeper water (150–350 m) was distinctly different (Fig. 5d). The available evidence indicates that bathymetric factors may affect the distribution of different *H. hirudinea* genotypes as well. The isolates of the clade common to Admiralty Bay and the Ross Sea were collected in Rothera from 150 to 230 m, whereas isolates yielding sequences specific to Rothera were only from 230 m (Fig. 5a and Online Resource 1). Although based on limited data, these results taken together suggest that bathymetric factors may influence the distribution of Antarctic foraminiferal genotypes. This possible importance of bathymetry is consistent with earlier observations highlighting water depth as one of the main parameters associated with major changes in foraminiferal morphotype assemblages in the Antarctic (e.g., Kennett 1968; Majewski 2005; Gooday et al. 2014).

On the other hand, we also found examples of foraminiferal genotypes with wide bathymetrical ranges. *Epistominella* sp. comprises sequences from the Ross Sea and Admiralty Bay, where they occur in shallow-water sites above ~100 m (Online Resource 1). They cluster with sequences obtained from isolate 3614 from the Weddell Sea (Pawlowski et al. 2007a), which was collected from a depth of 1080 m (Fig. 4f). This molecular similarity confirms the eurybathic character of this species. *Globocassidulina biora*, ranging in Rothera down to at least 515 m (Online Resource 1), is yet another example of a widespread Antarctic species with a broad bathymetric range. Its bathymetric distribution may be surprising, as *G. biora* is regarded as a shallow-water inhabitant compared with its deeper congener *Globocassidulina subglobosa* (e.g., Majewski and Pawlowski 2010). Consistent with our molecular data, however, is the fact that large subfossil specimens of *G. biora* displaying their characteristic "double" aperture have been collected along West Antarctica from several hundred meters of water depth (e.g., Fillon 1974; Kellogg and Kellogg 1987; Majewski and Anderson 2009). These results confirm gene flow between shallow- and deep-water Antarctic environments (Pawlowski et al. 2007a).

Dispersal potential and dispersal patterns among Antarctic biota

Repeated expansions of the Antarctic ice sheet to the shelf break during glacial cycles (The RAISED Consortium 2014), were thought to dramatically reduce the continuity of shallow-water habitats (Thatje et al. 2005), severely affecting benthic organisms, including foraminifera. On the other

hand, subsequent glacial retreat from shelf areas permitted long-distance dispersal of periglacial biota beyond refugia. Our evidence for the widespread presence of genotypes within all of the nine examined morphospecies confirms this pattern and suggests active gene flow between the Ross Sea and South Shetlands. High dispersal potential seems to be necessary to maintain such distribution. Although benthic foraminifera do not show strictly pelagic stages in their life cycle, their propagules do seem to possess considerable dispersal potential (Alve and Goldstein 2003, 2010). Their passive dispersal along coastal areas could be facilitated by the counterclockwise Antarctic Coastal Current (e.g., Hemery et al. 2012). It does not explain, however, the considerable variability in genetic structure in certain benthic foraminiferal morphospecies.

Distribution patterns of Antarctic biota, including meiobenthos, are far from being predictable (e.g., Brandão et al. 2010; Baird et al. 2012). Indeed, it appears that high dispersal potential is not always a key to understanding the distribution of various organisms. Around Antarctica, species with poor dispersal abilities do not always show strongly restricted ranges, and vice versa. For example, considerable dispersal over hundreds of kilometers was reported for the brittle star *Astrotoma agassizii* (Hunter and Halanych 2008), which lacks a pelagic larval stage, while circum-Antarctic ranges have been documented in the brooding sea spider *Nyphon australe* (Arango et al. 2011) and the benthic octopus *Pareledone turqueti* (Strugnell et al. 2012). However, in the last two examples, clear genetic structure was also detected, suggesting limited gene flow. On the other hand, organisms with potentially large dispersal capabilities, like the Antarctic scallop *Adamussium colbecki* with pelagic larvae and the vagile crinoid *Promachocrinus kerguelensis* with positively buoyant larvae, turned out to be more diverse than anticipated (Guidetti et al. 2006; Wilson et al. 2007; Hemery et al. 2012). In the case of *P. kerguelensis*, seven phylogroups have been identified within what was previously believed to constitute a single circum-Antarctic species (Hemery et al. 2012). To add to the complexity of Antarctic biogeographic patterns, significant genetic differentiation was also observed between different populations of the pelagic Antarctic toothfish *Dissostichus mawsoni* (Kuhn and Gaffney 2008) and the krill *Euphausia superba* (Zane et al. 1998; Jarman et al. 2002), illustrating incompletely understood restrictions to gene flow within the pelagic realm.

Despite these nuances, the broad distribution of nine out of 12 foraminiferal genotypes identified in this study appears to be a consequence of their considerable dispersal capabilities. Moreover, the majority of those foraminifera that show uniform genetic structure (Figs. 4a–f) could represent species that have repopulated the Antarctic shelf from single ancestral populations. On the other hand, the complex genetic structure documented for *G. biora* (Fig. 2g) only in part results from intra-specific polymorphism, as seen in the case of *T. earlandi*. This genetic complexity suggests a more complicated history for *G. biora*, possibly blending within the present-day, widely distributed population with genetic material from a number of populations isolated during past expansions of the Antarctic ice sheet, as was similarly proposed for the crinoid *P. kerguelensis* (Hemery et al. 2012). In the case of the two morphospecies that show genotypes restricted to single sampling areas, i.e., *H. hirudinea* and *Micrometula* sp., more studies are required to determine whether they represent true complexes of cryptic species. The possibility that they may be distinguished based on morphological characteristics requires more detailed study of additional material. It is also uncertain whether or not their genetic divergence occurred before or after the recolonization of Antarctic coastal waters.

Conclusions

Some coastal foraminiferal morphospecies are widely distributed in Antarctic waters. We examined genetically nine morphospecies that are present in South Shetlands (Admiralty Bay) and in the western Ross Sea (McMurdo Sound and Terra Nova Bay) separated by a distance of ~4500 km. For three of these morphospecies, we included specimens from Rothera (Marguerite Bay), which is located between the two main areas of interest. Our study, based on SSU and ITS rDNA sequence data, shows broad distribution of nine out of 12 foraminiferal genotypes. It suggests active gene flow between shallow-water habitats of the Ross Sea and South Shetlands, most likely as a consequence of considerable dispersal capabilities of foraminiferal propagules. This broad distribution may extrapolate to most coastal areas of West Antarctic, if not around the entire Antarctic continent.

The majority of foraminifera we studied that show uniform genetic structure could represent species that have repopulated the Antarctic shelf following repeated ice sheet expansions and retreats. The complex genetic structure documented for *G. biora* suggests blending of the present-day, widely distributed population with genes from a number of populations isolated during past expansions of the Antarctic ice sheet. This genetic complexity results only in part from intra-specific polymorphism, as in the case of *T. earlandi* that shows unusually high intra-individual genetic variability. Multiple genotypes within *H. hirudinea* and *Micrometula* sp. from Rothera suggest that bathymetry may influence distribution patterns along the West Antarctic shelf. On the other hand, *Epistominella* sp. and *G. biora* show uniform genotypes with

wide bathymetrical ranges, providing examples for eurybathy among Antarctic benthic foraminifera.

Acknowledgments We thank Frederic Sinniger and Simon Creer (CGS-67) for providing sediment samples from Rothera and Jackie Guiard and Emanuela Reo for technical assistance. We also thank the divers and field staff of Project B-043 for the McMurdo sampling, as well as Frederic Sinniger and Béatrice Lecroq for diving to collect samples from Admiralty Bay. This work was supported by Swiss National Science Foundation grant 31003A_140766, US National Science Foundation grant ANT0944646, Polish National Science Centre grant 2011/01/B/ST10/06956, and the G. and A. Claraz Donation. We would like to thank three anonymous reviewers for their helpful comments on this paper.

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